

# The effects of free-air CO<sub>2</sub> enrichment and soil water availability on spatial and seasonal patterns of wheat root growth

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## Abstract

Spring wheat [*Triticum aestivum* (L). cv. Yecora Rojo] was grown from December 1992 to May 1993 under two atmospheric CO<sub>2</sub> concentrations, 550 µmol mol<sup>-1</sup> for high-CO<sub>2</sub> plots, and 370 µmol mol<sup>-1</sup> for control plots, using a Free-Air CO<sub>2</sub> Enrichment (FACE) apparatus. In addition to the two levels of atmospheric CO<sub>2</sub>, there were ample and limiting levels of water supply through a subsurface drip irrigation system in a strip, split-plot design. In order to examine the temporal and spatial root distribution, root cores were extracted at six growth stages during the season at in-row and inter-row positions using a soil core device (86 mm ID, 1.0 m length). Such information would help determine whether and to what extent root morphology is changed by alteration of two important factors, atmospheric CO<sub>2</sub> and soil water, in this agricultural ecosystem. Wheat root growth increased under elevated CO<sub>2</sub> conditions during all observed developmental stages. A maximum of 37% increase in total root dry mass in the FACE vs. Control plots was observed during the period of stem elongation. Greater root growth rates were calculated due to CO<sub>2</sub> enhancement until anthesis. During the early vegetative growth, root dry mass of the inter-row space was significantly higher for FACE than for Control treatments suggesting that elevated CO<sub>2</sub> promoted the production of first-order lateral roots per main axis. Then, during the reproductive period of growth, more branching of lateral roots in the FACE treatment occurred due to water stress. Significant higher root dry mass was measured in the inter-row space of the FACE plots where soil water supply was limiting. These sequential responses in root growth and morphology to elevated CO<sub>2</sub> and reduced soil water supports the hypothesis that plants grown in a high-CO<sub>2</sub> environment may better compensate soil-water-stress conditions.

**Keywords:** CO<sub>2</sub>, FACE, climate change, root growth, *Triticum aestivum* L., water stress

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## Introduction

As atmospheric carbon dioxide concentration [CO<sub>2</sub>] increases, alterations of terrestrial ecosystems may occur (IPCC 1995). Attributing CO<sub>2</sub> effects on plants is more uncertain at the ecosystem level than on the leaf or single plant level because of interactions between components of the plant–soil system. Critical to the understanding of the consequences of global climate change for terrestrial ecosystems is the role of the root system and below-

ground processes in regulating plant responses to rising [CO<sub>2</sub>]. Because root growth patterns are more flexible than above-ground organs, a less genetically limited potential for taking up additional carbon in the roots compared with the above-ground biomass is expected (Thomas & Strain 1991).

A greater root system growing in high [CO<sub>2</sub>] is supposed to achieve an important sink for increasing fixed carbon in [CO<sub>2</sub>]-enriched agricultural and natural ecosystems. Numerous studies have shown that elevated [CO<sub>2</sub>] may result in increased root biomass for agronomic crops (reviewed by Rogers *et al.* 1994), root-length density,

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and root dry weight, particularly that of fine roots (i.e. Curtis *et al.* 1994; Prior *et al.* 1994a,b). Greater growth rates, root diameter, root volume, and root area also have been found in higher  $[\text{CO}_2]$  (i.e. Wong & Osmond 1991; Rogers *et al.* 1992, 1993; Wall *et al.* 1996). Thus, the net input of organ carbon to the soil increases and influences different soil processes (Diaz *et al.* 1993; Zak *et al.* 1993).

Growth and yield responses to elevated  $[\text{CO}_2]$  above and below have complex interactions with abiotic environmental factors. Moreover, the response of microbial dynamics in rhizosphere and soil, as well as biochemical turnover processes to elevated  $[\text{CO}_2]$ , may alter when interacting with temperature, soil water and nutrients (Lambers 1993; Kemp *et al.* 1994; Wood *et al.* 1994; Leavitt *et al.* 1996). Therefore, soil carbon storage due to elevated  $[\text{CO}_2]$  has been observed to modify differently. Some researchers found an increase in organic soil carbon (Schlesinger 1990; Trans *et al.* 1990; Casella & Soussana 1997). Conversely, soil C decreased in several studies (Kirschbaum 1995; Grant *et al.* 1998) or soil C inputs and outputs were more balanced out (Pinter *et al.* 1996). That's why more information about experimental unrestricted root growth as an important below-ground factor in ecosystems in elevated  $[\text{CO}_2]$  in interaction with soil water is required when predicting future  $[\text{CO}_2]$  and the 'missing sink' within the global carbon cycle (Norby 1994; Schimel 1995; Lal *et al.* 1998).

It has been repeatedly observed that increasing  $[\text{CO}_2]$  may be ameliorated by water stress (Rogers *et al.* 1994). This may happen because of the improved water status of the plant reflected by a lower ET and increased water-use efficiency in elevated  $[\text{CO}_2]$  (Owensby *et al.* 1997). On the other hand, high  $[\text{CO}_2]$  may induce a greater root system with improved root physiological and morphological characteristics that may counteract by facilitating nutrient acquisition when water stress inhibits the flux of soil water to the plant (Bassirirad *et al.* 1997; van Vuuren *et al.* 1997).

To examine the response of a wheat crop root system to global change a Free-air  $\text{CO}_2$  Enrichment (FACE) experiment was conducted under full-season  $[\text{CO}_2]$  supply and two irrigation levels. Objectives of the study were (i) to determine the seasonal course of root growth by quantifying the root dry mass (ii) to determine horizontal and vertical patterns of wheat root growth in  $[\text{CO}_2]$ -ambient compared with  $[\text{CO}_2]$ -enriched regimes, and (iii) to observe the response of wheat root growth to elevated  $[\text{CO}_2]$  in interaction with limited soil water.

## Materials and methods

### Experiment site and treatment description

A Free-Air  $\text{CO}_2$  Enrichment (FACE) experiment was carried out with hard red spring wheat [*Triticum aestivum*

(L. cv. Yecora Rojo)] at the University of Arizona Maricopa Agricultural Center located 50 km south of Phoenix, AZ (USA, 33.07 °N, 111.9 °W). The physical and chemical characteristics of the soil, which was classified as a Trix clay loam [fine-loamy, mixed (calcareous) hyperthermic Typic Torrifluvent], have been described elsewhere (Wall & Kimball 1993; Pinter *et al.* 1996). On 15 December 1992 wheat seeds were sown in flat beds at a 0.25-m-row spacing with a plant population of 130 plants  $\text{m}^{-2}$ ; 50% emergence occurred on 1 January 1993 and the crop was harvested on 21 May 1993. After sowing, FACE was conducted on site. Elevation of  $[\text{CO}_2]$  commenced with crop emergence on 1 January 1993, and terminated at the time of grain harvest 16 May 1993. Since crop emergence was the first Day of Year (DOY) in 1993, DOY was equivalent to days after emergence. Four 25-m diameter FACE rings were fumigated with  $\text{CO}_2$  for 24 h per day at rates required to maintain a  $[\text{CO}_2]$  of 550  $\mu\text{mol CO}_2 \text{ mol}^{-1}$  (FACE). Four control rings at ambient  $[\text{CO}_2]$  were monitored with a seasonal average  $[\text{CO}_2]$  of 370  $\mu\text{mol CO}_2 \text{ mol}^{-1}$  (Control). A complete description of the design, construction, and algorithms of the FACE exposure and monitoring system are provided by Hendrey (1993), Lewin *et al.* (1994) and Nagy *et al.* (1994).

The experimental design was a strip, split-plot with two levels of the main treatment,  $[\text{CO}_2]$ , replicated four times. Each of the eight circular main plots was split into two semicircular subplots to test the effect of two different irrigation amounts on wheat response to  $[\text{CO}_2]$ . All plots were watered throughout the growing season using a subsurface drip irrigation system installed 0.18–0.25 m below the soil surface with 0.3-m spacing between emitters along the tubes. One half of each main plot was irrigated at a target rate based on 100% replacement of potential evapotranspiration (Wet), and the other half was irrigated at a target rate of 50% (Dry). The subplots were irrigated in strips that extended across a main plot replicate. Thus, it was necessary to impose the same irrigation treatment on the same subplot side for each replicate. The Wet and Dry irrigation treatments were alternated over the four main plot replicates, such that two replicates had a Wet and Dry treatment on the northern side of the main plot, while the other two had a Wet and Dry treatment on the southern side. Preseason rainfall was above normal during 1992–93. Therefore, the water supply of the Dry treatment was similar to Wet treatment, including an initial water application for crop establishment (317 mm) late in December 1992 until the onset of the differential irrigation (DOY 060). Cumulative irrigation totals between crop emergence and harvest were 600 mm for the Wet treatment and 275 mm for the Dry treatment. Volumetric soil water contents were measured using Time-Domain-Reflectometry (TDR) and a neutron scattering equipment installed into soil at

beginning of the experiment on 18 December 1992. Average daily ET was determined by measuring the change in soil water over a period of time and calculating the soil water balance equation (Kimball *et al.* 1995; Hunsacker *et al.* 1996).

Except for using drip rather than flood irrigation, all agronomic practices were in accordance with local cultural production methods. All treatments received the same amount of fertiliser during the growing season. The total amount of nitrogen and phosphorus were applied at a rate of 277 kg ha<sup>-1</sup> and 44 kg ha<sup>-1</sup>, respectively, through the drip irrigation system (Pinter *et al.* 1996).

#### Root sampling and analysis

Root cores were taken to a 0.3-m soil depth at three-leaf stage (DOY 016), 0.6-m depth at tillering (DOY 036), and 1.0-m depth at stem elongation, anthesis, dough development, and post harvest (DOY 063, 092, 113, 159, respectively) using a gas-engine-driven soil core device (Eijkelkamp Agrisearch Equipment, Cobra Model # 248). Two in-row and one inter-row root cores (86 mm ID) were collected on each sampling date except at three-leaf stage when three cores were collected at the in-row position. The numbers of replicate blocks were four at three-leaf stage, tillering, stem elongation, and anthesis; but, unfortunately, reduced to two at dough development and post harvest because of technical reasons. Cores were divided into 0.15-m sections from 0 to 0.60-m soil depth, and into 0.20-m sections from 0.60 to 1.0-m depth. During three-leaf stage and tillering, samples from the top-0.15-m core were divided additionally in 0–0.05 and 0.05–0.15 m sections. Root core samples were frozen (–14 °C) until processed. Root and organic-debris materials in each section of a core were elutriated from the soil with a hydropneumatic elutriation system (Gillison's Variety Fabrications Inc., Model GVF-13000, Benzonia, MI, USA, Gillison 1990). Live roots were separated from organic debris material manually (intact, white-coloured roots), oven dried for two days at 68 °C, desiccator cooled, and weighed.

#### Data analysis

Statistical analysis was performed using the general models procedure (SAS 1985). Statistical differences and least-square means comparisons were performed for each DOY, core position (in-row, interrow), and soil depth. Data were analysed as randomised block for the main effect (CO<sub>2</sub>). The main effect was split into nonrandomized irrigation (H<sub>2</sub>O) effect. The error used for evaluating the main CO<sub>2</sub> effect and effect of H<sub>2</sub>O were (CO<sub>2</sub> × REP) and (H<sub>2</sub>O × REP), respectively. The error terms (REP × CO<sub>2</sub>) and (REP × H<sub>2</sub>O), respectively, were pooled to the

residual error term, if (Rep × CO<sub>2</sub>) and (Rep × H<sub>2</sub>O), respectively, were insignificant ( $P > 0.15$ ). The error used for evaluating the interaction term (CO<sub>2</sub> × H<sub>2</sub>O) was the residual mean-square error.

A multiplicative linear regression model was used for estimating the mean influences of CO<sub>2</sub> and H<sub>2</sub>O treatment on root biomass and root growth during the phase of growth followed by senescence:

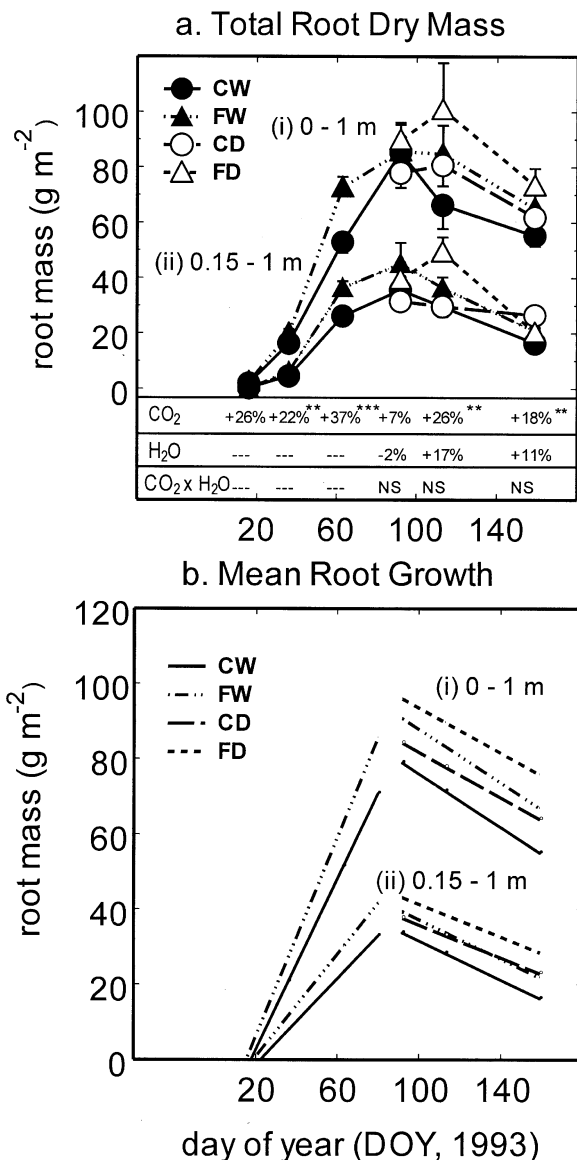
$$R_{DW} = \beta_0 + \beta_1(\text{DOY}) + \beta_2(\text{DOY } \theta_c) + \beta_3(\text{DOY } \theta_l) + \beta_4(\theta_c) + \beta_5(\theta_l),$$

where the dependent variable ( $R_{DW}$ ) is the root dry weight (g m<sup>-2</sup>), DOY is the independent variable,  $\beta_0$  is the intercept of the regression model, and  $\beta_1$  the time dependent part of the root growth rate (g m<sup>-2</sup> d<sup>-1</sup>). The root growth rate  $\beta_1$  quantifies the unit mass increment in root mass per calendar day. The slopes  $\beta_2$  and  $\beta_3$  correspond to a changing growth rate due to [CO<sub>2</sub>] and H<sub>2</sub>O (g m<sup>-2</sup> d<sup>-1</sup>), respectively. Thus,  $\beta_2$  characterizes the contribution of different [CO<sub>2</sub>] on growth and senescence rate, whereas  $\beta_3$  characterizes the contribution of different H<sub>2</sub>O on root-senescence rate. The variables  $\beta_4$  and  $\beta_5$  express constant differences in the mean  $R_{DW}$  over time caused by CO<sub>2</sub> and H<sub>2</sub>O. Variables  $\theta_c$  and  $\theta_l$  symbolize the [CO<sub>2</sub>] and irrigation (H<sub>2</sub>O) treatments, respectively.  $\theta_c$  equals 1 if [CO<sub>2</sub>] = 550 µmol mol<sup>-1</sup>, and 0 if ambient [CO<sub>2</sub>].  $\theta_l$  equals 1 if H<sub>2</sub>O = Wet, and 0 if H<sub>2</sub>O = Dry. Prior to any regression analysis, the analysis of variance was performed to determine the significant variables of the regression model according to the specific strip, split-plot design of the experiment. Insignificant variables were excluded from the regression model.

## Results

#### Seasonal trend in $R_{DW}$

No significant two-way (CO<sub>2</sub> × H<sub>2</sub>O) interactions were observed on  $R_{DW}$  at six growth stages averaged over two sample positions in 0–1.0-m soil depth. This indicates that CO<sub>2</sub> and H<sub>2</sub>O effects were predominant, and total  $R_{DW}$  was stimulated due to FACE under both Wet and Dry conditions for most of the growing season (Fig. 1a). An initial trend of higher  $R_{DW}$  in response to FACE occurred at the three-leaf stage in row (26%,  $P \leq 0.15$ , Table 1). Significant main CO<sub>2</sub> effects for  $R_{DW}$  in-row and inter-row positions on average were observed at tillering (22%,  $P \leq 0.05$ ), at stem elongation (37%,  $P \leq 0.01$ ), at dough development (26%,  $P \leq 0.05$ ) and at post harvest (18%,  $P \leq 0.05$ ). These main CO<sub>2</sub> effects were also significant in the 0.15–1.0-m soil layer except for three-leaf stage and post harvest (Fig. 1a). A considerable high amount of 70% additional  $R_{DW}$  due to the FACE



**Fig. 1** Spring wheat root growth by day of year (DOY) under 370 (Control) and 550 (FACE)  $\mu\text{mol mol}^{-1}$  [CO<sub>2</sub>], wet and dry soil water regimes for the Control-Wet (CW), FACE-Wet (FW), Control-Dry (CD), and FACE-Dry (FD) treatments. (a) Seasonal course of mean root biomass and standard errors ( $\text{g m}^{-2}$ ), where DOY 016, 036, 063, 092, 113, 159 correspond to three-leaf stage, tillering, stem elongation, anthesis, dough development and final harvest, respectively; averaged over two sample positions, and summarized over (i) all investigated soil depths (0–1.0 m), and (ii) the soil layers below 0.15 m (0.15–1.0 m). Maximal investigated soil depth were at three-leaf stage 0–0.3 m and at tillering 0–0.6 m. Shown also are main treatment effects of the 0–1-m profile for CO<sub>2</sub>, H<sub>2</sub>O, and CO<sub>2</sub>–H<sub>2</sub>O interaction effects (\*\*, \*, \* for  $P \leq 0.01$ ,  $P \leq 0.05$ ,  $P \leq 0.1$ ). Differential irrigation was provided from DOY 060–134. (b) Mean root growth calculated using multiplicative linear regressions during the vegetative (DOY 16–92) and reproductive periods (DOY 92–159) of plant development, averaged over two sample positions, summarized over (i) all soil depths (0–1.0 m), and (ii) soil layers below 0.15 m (0.15–1.0 m).

application was found in the inter-row position ( $P \leq 0.05$ ) compared to a 18% increase of  $R_{\text{DW}}$  in row ( $P > 0.1$ , Table 1). This supports the idea that during the early growth stages plants grown in higher [CO<sub>2</sub>] partitioned assimilates preferentially in horizontal direction by more developing and branching lateral roots.

Differential water supply was provided from DOY 060 to DOY 134. Differences in soil water content and separation in soil matrix potential were recorded at DOY 068 (Hunsacker *et al.* 1996; Wall *et al.* 1998a,b, pers. com.). Therefore, significant alteration in  $R_{\text{DW}}$  due to Dry treatments did not occur before anthesis (DOY 092). Data averaged from anthesis through post harvest showed a significant H<sub>2</sub>O effect on  $R_{\text{DW}}$  by 9% due to the Dry treatments ( $P \leq 0.1$ , Fig. 1a). In the inter-row space even 23% more ( $P \leq 0.05$ ) occurred in Dry ( $P \leq 0.05$ ) than in Wet for the same period (Table 1). A significant two-way (CO<sub>2</sub>  $\times$  H<sub>2</sub>O) interaction effect occurred only in the inter-row sample space during dough development ( $P \leq 0.05$ , Table 1). Thus, in the averaged 0–1.0-m soil depths of the dry-inter-row plots, plants in FACE reached the greatest  $R_{\text{DW}}$ , whereas  $R_{\text{DW}}$  in well watered FACE plots did not differ significantly from the Control treatment (Table 1).

#### Root growth and senescence rate

No significant (CO<sub>2</sub>  $\times$  H<sub>2</sub>O) interaction effects were observed; thus, the (DOY  $\times$  CO<sub>2</sub>  $\times$  H<sub>2</sub>O) term was excluded from the regression model. Regressing wheat mass with DOY gave significant linear slopes for FACE and Control (Fig. 1b, Table 2) that characterize the phase of root growth and senescence. The mean root growth rate ( $\beta_1$ ) shows that the  $R_{\text{DW}}$  of all treatments increased from three-leaf stage (DOY 016) until anthesis (DOY 092) by  $1.14 \text{ g m}^{-2} \text{ d}^{-1}$  in 0–1.0-m soil depth (Table 2). From anthesis to dough development (DOY 113) root growth and root senescence were similar. During dough development through post harvest a greater proportion of roots died and  $R_{\text{DW}}$  decreased by an average of  $30 \text{ g m}^{-2} \text{ d}^{-1}$ .

The mean influence of [CO<sub>2</sub>] and H<sub>2</sub>O on  $R_{\text{DW}}$  was estimated for the two growth periods by calculating growth and senescence rates ( $\beta_2$ ,  $\beta_3$ ). A 15.8% increase of  $R_{\text{DW}}$  ( $\beta_1 = 1.14 \text{ g m}^{-2} \text{ d}^{-1}$ ) during the vegetative growth occurred due to FACE, as indicated by a growth rate,  $\beta_2$  of  $0.18 \text{ g m}^{-2} \text{ d}^{-1}$  in 0–1.0 m (Table 2). A growth rate,  $\beta_2$  of  $0.11 \text{ g m}^{-2} \text{ d}^{-1}$  was observed in 0.15–1.0-m depth. Thus, 61% of the total additional  $R_{\text{DW}}$  induced by FACE grew in lower depths. There was a trend of constant differences of  $2.31 \text{ g m}^{-2} \text{ d}^{-1}$  between root growth rates in FACE and Control in the 0–0.15-m soil depth ( $\beta_4$ , Table 2). This indicates that the increase in root growth in FACE during the vegetative period occurred to a high extent below the 0.15-m depth ( $\beta_4 = \text{NS}$ ). No significant CO<sub>2</sub> effect on senescence rate (DOY 092–159,  $\beta_2$ , Table 2) was observed.



**Table 1** Seasonal course by day of year (DOY) in 1993 and corresponding growth stage of mean root biomass ( $\text{g m}^{-2}$ ) of spring wheat within the top 1-m soil depth<sup>1</sup> for different sample positions grown under 370 (Control) and 550 (FACE)  $\mu\text{mol mol}^{-1}$   $[\text{CO}_2]$ , wet and dry soil water regimes; also shown are main treatment effects<sup>2</sup> for  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ , and  $\text{CO}_2 \times \text{H}_2\text{O}$  interaction effects

Position	Treatment	Growth stage, DOY					
		three-leaf stage 016	tillering 036	stem elongation 063	anthesis 092	dough development 113	post harvest 159
in row	Control Wet	3.57	23.43	67.98	123.70	87.84	88.84
	FACE Wet	4.95	27.76	90.39	117.67	122.14	104.25
	Control Dry <sup>(3)</sup>	–	–	–	105.39	113.74	81.66
	FACE Dry	–	–	–	123.77	132.48	97.46
	$\text{CO}_2$	NS	NS	***	NS	NS	**
	$\text{H}_2\text{O}$	–	–	–	NS	NS	NS
	$\text{CO}_2 \times \text{H}_2\text{O}$	–	–	–	NS	NS	NS
inter row	Control Wet	–	8.15	44.37	46.90	45.96	24.82
	FACE Wet	–	13.84	55.83	53.62	47.29	31.04
	Control Dry	–	–	–	52.35	47.59	40.58
	FACE Dry	–	–	–	55.85	65.55	35.71
	$\text{CO}_2$	–	**	NS	NS	*	NS
	$\text{H}_2\text{O}$	–	–	–	NS	*	**
	$\text{CO}_2 \times \text{H}_2\text{O}$	–	–	–	NS	**	NS

<sup>1</sup>soil depth at three-leaf stage was 0–0.3 m, and at tillering 0–0.6 m; <sup>2</sup>\*\*\*, \*\*, \* for  $P \leq 0.01$ ,  $P \leq 0.05$ ,  $P \leq 0.1$ ; <sup>3</sup>differential irrigation was provided from DOY 060–134.

**Table 2** Estimates of treatment-induced changes to mean root growth in different soil layers by applying a linear regression model for the vegetative (DOY 16–92) and the reproductive growth (DOY 92–159), where  $\beta_0$  is the intercept parameter of the regression model,  $\beta_1$  the time-dependent part of the root growth rate,  $\beta_2$  and  $\beta_3$  correspond to changing growth rates due to higher  $[\text{CO}_2]$  and water stress, respectively, and  $\beta_4$  and  $\beta_5$  express constant differences in the mean root biomass over time caused by  $\text{CO}_2$  and irrigation, respectively

Depth [m]	Vegetative growth (DOY16–92)			Generative growth (DOY 92–159)		
	0–0.15	0.15–1.00	0–1.00	0–0.15	0.15–1.00	0–1.00
$\beta_0$ <sup>(1)</sup>	–9.93 S <sup>(2)</sup>	–12.74 S	–20.27 S	53.85 S	57.78 S	111.63 S
$\beta_1(\text{DOY})$	0.64 S	0.57 S	1.14 S	–0.08	–0.22 S	–0.30 S
$\beta_2(\text{DOY } \theta_C)$	NS	0.11 S	0.18 S	( $P \leq 0.12$ )	NS	NS
$\beta_3(\text{DOY } \theta_I)$	– <sup>3</sup>	–	–	NS	–0.042 S	–0.056 ( $P \leq 0.1$ )
$\beta_4(\theta_C)$	2.31 ( $P \leq 0.2$ )	NS	NS	6.22 ( $P \leq 0.1$ )	5.6 ( $P \leq 0.2$ )	11.82 S
$\beta_5(\theta_I)$	–	–	–	NS	NS	NS
$P_{\text{Regression}} < F^{(2)}$	S	S	S	( $P \leq 0.05$ )	S	S
$R^2$	0.86	0.78	0.86	0.10	0.51	0.37

<sup>1</sup>Parameters of the linear regression:  $R_{\text{DW}} = \beta_0 + \beta_1(\text{DOY}) + \beta_2(\text{DOY } \theta_C) + \beta_3(\text{DOY } \theta_I) + \beta_4(\theta_C) + \beta_5(\theta_I)$

$\theta_C = \{1 \text{ if } [\text{CO}_2] = 550 \mu\text{mol mol}^{-1}; 0 \text{ else } \theta_I = \{1 \text{ if the irrigation treatment is Wet; } 0 \text{ else; where the variables DOY, } \theta_C \text{ and } \theta_I \text{ symbolize day of the year, the } \text{CO}_2 \text{ treatment; and the Irrigation treatment, respectively. Two way interactions } (\text{CO}_2 \times \text{H}_2\text{O}) \text{ were insignificant } (P > 0.15) \text{ and from regression model excluded.}$

<sup>2</sup>Results from  $t$ -test of the regression parameter, and from  $F$ -test for the effect tested in the variance analysis are shown with S for all significant effects ( $P \leq 0.01$ ), for all less significant effects  $p$  ( $0.01 \leq P \leq 0.2$ ) is given, else NS.

<sup>3</sup>Differential irrigation was provided from DOY 060–134.

Therefore, the difference of  $11.82 \text{ g m}^{-2} \text{ d}^{-1}$  root dry mass between FACE and Control treatments after anthesis until post harvest for the whole 1.0-m profile remained significant constant ( $\beta_4$ , Table 2). Main  $\text{H}_2\text{O}$  effects are expressed by significant senescence rates ( $\beta_3$ ) of  $-0.056$  and  $-0.042 \text{ g m}^{-2} \text{ d}^{-1}$  for the 0–1.0-m and 0.15–1.0-m soil

depth, respectively, and also by nonsignificant  $\beta_5$  (Table 2). This means that soil water delayed the decrease of  $R_{\text{DW}}$  during senescence under dry soil conditions (Fig. 1b), and greater  $R_{\text{DW}}$  at harvest were observed in the FACE- and Control-Dry plots (Fig. 1a). FACE-Dry treatment induced an increase of measured  $R_{\text{DW}}$  until dough

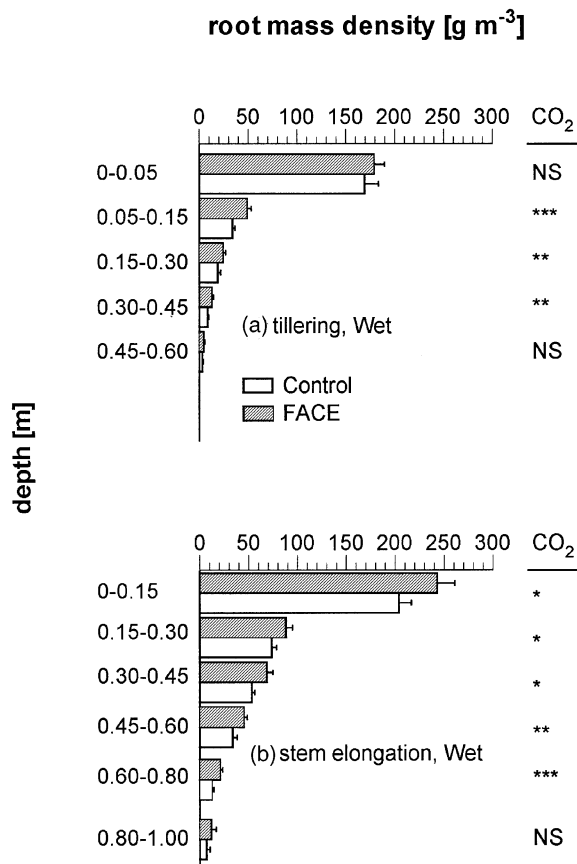


Fig. 2 Distribution of root mass density and standard errors along the vertical 0–1.0-m soil profile for wheat grown under Control and FACE conditions and ample soil moisture (Wet) at (a) tillering (b) stem elongation.

development, and then a substantial decrease of  $29 \text{ g m}^{-2}$  to post harvest (Fig. 1a). Thus, at post dough development, a daily  $R_{DW}$  reduction by  $0.63 \text{ g m}^{-2} \text{ d}^{-1}$  in FACE Dry was higher than in Control Dry ( $0.39 \text{ g m}^{-2} \text{ d}^{-1}$ ), in FACE Wet ( $0.37 \text{ g m}^{-2} \text{ d}^{-1}$ ), and in Control Wet ( $0.42 \text{ g m}^{-2} \text{ d}^{-1}$ ).

#### Development of the vertical root profile and spatial distribution of $R_{DW}$

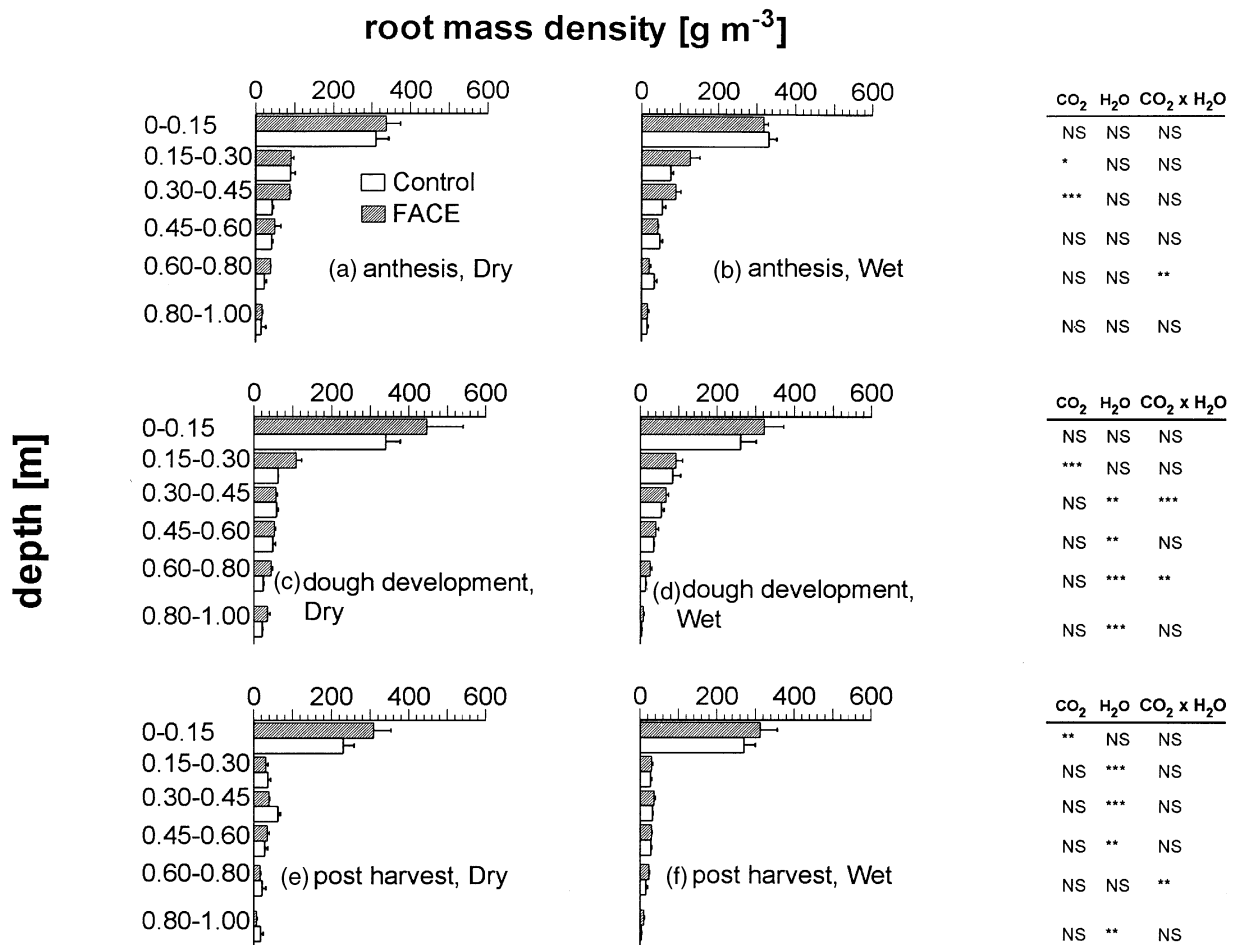
The vertical distribution of the  $R_{DW}$  from in-row and inter-row sample positions on average was depicted in Figs 2 and 3. In general, the greatest  $R_{DW}$  was observed within the top-0.15-m soil layer. During tillering (DOY 036) 69% of roots grew in 0–0.05-m depth. But, in this soil layer  $R_{DW}$  remained unchanged when impacted with high  $[\text{CO}_2]$ . However, 30–43% significant more roots due to FACE were recorded between 0.05 and 0.45 m in lower depths ( $P \leq 0.01$ , Fig. 2a). Later, during stem elongation, FACE plots had 19–20% more  $R_{DW}$  in the top-0.45-m soil layers than Controls ( $P \leq 0.1$ ). Further, in the well rooted

0–0.15 soil layer, where over 50% of the total  $R_{DW}$  were measured initial significant differences in  $R_{DW}$  between FACE and Control ( $P \leq 0.1$ ) were recorded during this growth stage, 63 days after emergence (Fig. 2b). In comparison, during this growth period at lower depths (0.45–0.80 m), between 36 and 73% more  $R_{DW}$  was formed due to FACE ( $P \leq 0.01$ ). After this, with the beginning of senescence a significant  $\text{CO}_2$  effect in  $R_{DW}$  occurred in ever shallower depths. We observed 75% more  $R_{DW}$  due to FACE at 0.30–0.45-m depth, DOY 092 ( $P \leq 0.01$ ); 55% at 0.15–0.30 m, DOY 113 ( $P \leq 0.01$ ); and 28% at 0–0.15 m, DOY 159 ( $P \leq 0.05$ ), respectively (Fig. 3b,d,f).  $R_{DW}$  in the 0.8–1.0-m soil layer were greater in FACE than in Control during dough development and post harvest, but not significant (Fig. 3d,f).

Soil water effects were highly significant during grain-filling, below 0.3-m depth until the end of season (Fig. 3). Significant ( $\text{CO}_2 \times \text{H}_2\text{O}$ ) interactions were found in 0.3–0.45, and 0.6–0.8-m depths during dough development, DOY 113, and occurred at anthesis, DOY 092 and post harvest, DOY 159, in 0.6–0.8-m soil depth. In addition, above mentioned ( $\text{CO}_2 \times \text{H}_2\text{O}$ ) effects were recognized in the inter-row space at dough development (Table 1). Following this indications, we can see that the more production of  $R_{DW}$  in the FACE-Wet plots until the peak of root biomass during anthesis (Fig. 1a) occurred mainly in the 0.15–0.45-m soil depths (Fig. 4a) in the in-row soil space and only partially in the inter-row section (Fig. 4b). Conversely, in the FACE-Dry treatments  $R_{DW}$  increased until dough development (Fig. 1a). Additional  $R_{DW}$  in this treatment was preferentially developed in the inter-row space (Fig. 4d) or in lower depth (0.6–1.0 m) in row (Fig. 4c). Water stress in ambient  $[\text{CO}_2]$  induced a root growth pattern with additional biomass at 0.6–1.0-m depths in row (Fig. 4c) and at 0.8–1.0-m depth (Fig. 4d).

#### Discussion

Seasonal course of wheat  $R_{DW}$  showed a definitive pattern, with a peak  $R_{DW}$  around anthesis (Fig. 1a). The amount of  $R_{DW}$  was similar to findings with wheat (*Triticum aestivum* L. cv. Newtons) in other field studies (Chaudhuri *et al.* 1990). Root mass profiles over depth (Figs 2,3) showed an exponential distribution, and coincides with other findings (Gerwitz & Page 1974). Soil water content for all treatments remained near field capacity until DOY 015 and was not significant different until DOY 077 when the Dry treatments reached about 80% soil water depletion before another irrigation occurred while soil water of the Wet treatments were maintained to field capacity (Hunsacker *et al.* 1996). Thus, effects of water stress were only significant during reproductive growth. During dough development and harvest 17% more  $R_{DW}$  in the Dry treatments was



**Fig. 3** Distribution of root mass density and standard errors along the vertical 0–1.0-m soil profile for wheat grown under Control-Wet (CW), FACE-Wet (FW), Control-Dry (CD), and FACE-Dry (FD) conditions during: anthesis (a) Dry (b) Wet; dough development (c) Dry (d) Wet; post harvest (e) Dry (f) Wet.

observed while 13% decrease of the above-ground biomass occurred under limited soil water (Pinter *et al.* 1996). This effect of disproportional above- and below-ground plant growth under dry soil conditions have also been reported elsewhere for other agronomic crops (i.e. Stone *et al.* 1976; Meyer *et al.* 1990; McMichael & Quisenberry 1993).

The relative increase in  $R_{DW}$  due to elevated [CO<sub>2</sub>] was 22% for the whole season and reached a maximal effect of 37% at stem elongation (DOY 063, Fig. 1a). In general, these results agree with those obtained from many other [CO<sub>2</sub>]-enrichment studies on different crops (Rogers *et al.* 1994). The [CO<sub>2</sub>] response of root dry mass for wheat [*Triticum aestivum* (L.) cv. 'Yecora Rojo'] was markedly lower than that obtained for cotton [*Gossypium hirsutum* (L.) 'Delta Pine 77'] in previous FACE experiments where in 1990, an 82% increase for taproot dry weight occurred (Rogers *et al.* 1993), and in 1991 a 62% increase for taproot dry weight, and 87% for lateral root dry weight, respectively, were observed (Prior *et al.* 1994b,c). Root

dry weight density values of cotton fine roots during vegetative growth were significantly greater under [CO<sub>2</sub>] enrichment in both years. In 1990 and 1991 root dry weight density increased between 35% at in-row position and 109% at the inter-row zone, 0.5 m from row centre (Prior *et al.* 1994a). During reproductive stage in 1990 root dry weight density enhanced by 29% in row, and in 1991 by maximal 54% and 50% at 0.25-m and 0.50-m inter-row positions.

A 30% greater wheat  $R_{DW}$  from tillering to stem elongation on average (Fig. 1a) and a 14% higher shoot growth due to FACE (Pinter *et al.* 1996) during this period of nonlimited soil water, emphasize the substantially higher sink potential in below-ground than in above-ground plant parts. This phenomenon of increasing root/shoot ratio was also observed in other species, for example in *Pinus palustris* Mill. due to higher [CO<sub>2</sub>] in open top chambers (Prior *et al.* 1997) and in *Lolium perenne* in FACE (Jongen *et al.* 1995). In the same FACE experiment *Trifolium repens* had equal or smaller root/shoot ratios (Hebeisen

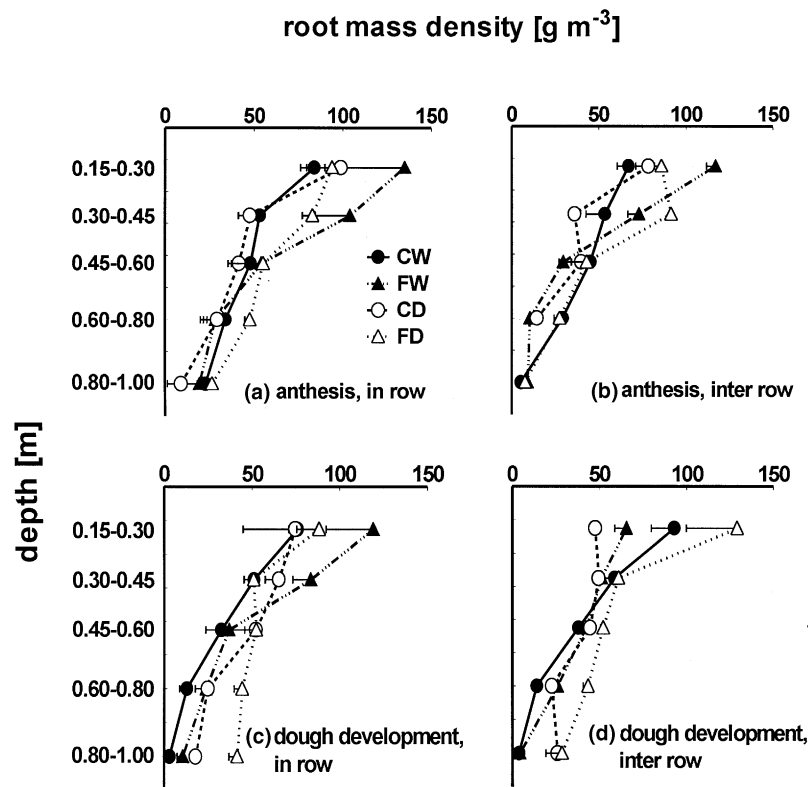


Fig. 4 Spatial root mass distribution of wheat in the vertical 0.15–1.00-m soil profile and two sample positions (in row and inter row) grown under Control-Wet (CW), FACE-Wet (FW), Control-Dry (CD) and FACE-Dry (FD) conditions during: anthesis (a) in row (b) inter row; dough development (c) in row (d) inter row.

*et al.* 1997). It was concluded that increases in root/shoot ratios under elevated  $[\text{CO}_2]$  could be due to a relative stronger N limitation. In *Citrus Aurantium* Idso & Kimball (1992) found no significant differences between ambient and  $[\text{CO}_2]$ -enriched trees regarding the fine-root biomass to the trunk cross-sectional area ratio. Based on this and other results they derived the assumption that increases in above- and below-ground growth are approximately equivalent for crops where the primary yield component is produced above ground (Idso *et al.* 1988).

Elevated  $[\text{CO}_2]$  stimulated  $R_{\text{DW}}$  at different soil depths during vegetative growth (Figs 2, 3). Chaudhuri *et al.* (1990) reported that wheat roots grown under  $[\text{CO}_2]$  enrichment penetrated to the maximal depth faster than the roots of plants grown at an ambient level. In the current study, considering the  $R_{\text{DW}}$ , this question of a faster and deeper soil exploration by roots due to higher  $[\text{CO}_2]$  can not be answered completely. In earlier findings of this experiment, Wechsung *et al.* (1995) reported about no significant estimated maximum rooting depth ( $D_{\text{max}}$ ) by regressing sampling depth with root mass except for the inter-row space, early in the season. Including the irrigation effect aspect in our study, at each particular growth stage from tillering through post harvest, significant  $\text{CO}_2$  effects at different depths occurred above the lowest depth increment of  $R_{\text{DW}}$  (Figs 2, 3). At the 0.8–1.0-m depth, more  $R_{\text{DW}}$  due to FACE was observed in tendency ( $P > 0.2$ ) which may suggest that FACE roots

grew deeper than Control roots. In a companion paper of this experiment, Wall *et al.* (1996) reported a significant  $\text{CO}_2$  effect on root length density ( $\text{km m}^{-3}$ ) at stem elongation for the 0.8–1.0-m depth that supports the idea of a deeper growing FACE root system. Roots also had significant lower length root densities ( $\text{g m}^{-3}$ ) at this depth from the three-leaf stage until stem elongation (Wall *et al.* 1996) which indicates a more ramified or branched root system in FACE vs. Control. Thus, we conclude that FACE roots had a better potential for assimilate allocation because they proliferated, ramified and branched quicker within a soil layer. This type of root development may infer an advantage in acquiring nutrients and water due to the increased number of young fine roots.

Bingham & Stevenson (1993) have proposed that carbohydrates are involved in regulating the initiation and development of lateral roots and the rate of extension of emerged roots, but they qualified that carbohydrates constitute only one component of the overall control mechanism. Consistent with this hypothesis, we found during the growing season subsequent respond of root growth to higher  $[\text{CO}_2]$  that was partially altered by soil water. The first significant respond of root biomass to high  $[\text{CO}_2]$  was found at tillering. The increase of  $R_{\text{DW}}$  was greater and significant only at the inter-row section compared with the in-row position (Table 1). Despite root growth was greatest in the first 0.15 m of the soil profile,



remarkable alterations of root distribution due to elevated  $[\text{CO}_2]$  were measured in lower depths (Fig. 2a). Significant greater  $R_{\text{DW}}$  under elevated  $[\text{CO}_2]$  occurred during stem elongation at 0–0.8-m soil depths (Fig. 2b). Again, additional root mass was found mainly at lower inter-row position (0.15–0.8 m). During grain filling, when soil water was limited, plants responded by increasing their  $R_{\text{DW}}$  to fulfil the demand of water and nutrients. While the Dry treatment in ambient  $[\text{CO}_2]$  more  $R_{\text{DW}}$  produced within the lower in-row section, plants grown under FACE increased root production also considerably within the inter-row position. It seems obvious, that greater horizontal root growth early in the season and continued higher assimilate allocation below ground due to  $[\text{CO}_2]$  enhancement enabled the plants to better expand their root system within the less used inter-row soil space when water stress occurred. In contrast, FACE application and sufficient soil water preferentially induced greater  $R_{\text{DW}}$  at the upper-in-row soil space during anthesis (Fig. 4a). In this soil compartment  $R_{\text{DW}}$  remained high until dough development (Fig. 4c). These sequential responses in root growth and spatial root distribution to elevated  $[\text{CO}_2]$  and low soil water support the idea, that plants grown in a high  $\text{CO}_2$  environment may better compensate for soil-water-stress conditions (Rogers *et al.* 1994) by improving their root system.

Root growth and senescence rates were estimated from changes in root dry mass. These parameters have been determined in an attempt to better describe the below-ground growth in spring wheat. Root growth rates allowed us to follow the seasonal course of life roots representing the physiological active part of the root system and, further, to select the  $\text{CO}_2$  and  $\text{H}_2\text{O}$  initiated effects on root growth. As root mortality was not determined in this study the total root growth can not be derived from available data and the allocation of assimilates below ground might be under or over estimated. However, the observed amount of dead roots during the manual selection of life roots was very small during the vegetative growth so that it may be neglected. A more complete analysis of the effect of root mortality in a FACE–nitrogen–wheat study is currently in progress, but the results to date indicate that elevated  $[\text{CO}_2]$  had no effect on dead root mass. However, data suggest that nitrogen stress increased the rate of root mortality during dough development. Considering the clear increase of  $R_{\text{DW}}$  in the Dry treatments until dough development in the current study, and the greater reduction of life roots after this (Fig. 1a) a higher C allocation rate to the roots and greater root mortality in the end of season could be assumed.

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### References

- Bassirirad H, Reynolds JF, Virginia RA, Brunelle MH (1997) Growth and root  $\text{NO}_3$  and  $\text{PO}_4^{3-}$  uptake capacity of three desert species in response to atmospheric  $\text{CO}_2$  enrichment. *Australian Journal of Plant Physiology*, **24**, 353–358.
- Bingham IJ, Stevenson EA (1993) Control of root growth: effects of carbohydrates on the extension, branching and rate of respiration of different fractions of wheat roots. *Physiologia Plantarum*, **88**, 149–158.
- Casella E, Soussana JF (1997) Long-term effects of  $\text{CO}_2$  enrichment and temperature increase on the carbon balance of an emperate grass sward. *Journal of Experimental Botany*, **48**, 1309–1321.
- Chaudhuri UN, Kirkham MB, Kanemasu ET (1990) Root growth of winter wheat under elevated carbon dioxide and drought. *Crop Science*, **30**, 853–857.
- Curtis PS, Zak LR, Dregitzer KS, Teeri JA (1994) Above and below-ground responses of *Populus grandidentata* to elevated atmospheric  $\text{CO}_2$  and soil N availability. *Plant and Soil*, **165**, 45–51.
- Diaz S, Grime JP, Harris J, McPherson E (1993) Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. *Nature*, **364**, 616–617.
- Gerwitz A, Page ER (1974) An empirical mathematical model to describe plant root systems. *Journal of Applied Ecology*, **11**, 773–783.
- Gillison's Variety Fabrication, Inc. (1990) *Basic Operation of the Hydropneumatic Root Washer*, p. 3033. Gillison's Variety Fabrication, Inc. Benzonia, MI.
- Grant RF, Izaurralde RC, Nyborg M, Mahli SS, Solberg ED, Jans-Hammermeister D (1998) Modelling tillage and surface residue effects on soil C storage and ambient vs. elevated  $\text{CO}_2$  and temperatures in ECOSYS. In: *Soil Processes and the Carbon Cycle* (eds Lal R, Kimble J, Follett RF, Stewart BA), pp. 1–9. CRC Press, Boca Raton, FL.
- Hebeisen T, Lüscher A, Zanetti S, Fischer BU, Hartwig UA, Frehner M, Hendrey GR, Blum H, Nosberger J (1997) Growth response of *Trifolium repens* L. & *Lolium perenne* L. as monocultures and bi-species mixtures to free air  $\text{CO}_2$  enrichment and management. *Global Change Biology*, **3**, 149–160.
- Hendrey GR (1993) FACE Free-Air  $\text{CO}_2$  Enrichment for Plant Research in the Field. CRC Press, Boca Raton, FL.
- Hunsacker DJ, Kimball BA, Pinter PJ Jr, LaMorte RL, Wall GW (1996) Carbon dioxide enrichment and irrigation effects on wheat evapotranspiration and water use efficiency. *American Society of Agricultural Engineering*, **39**, 1345–1355.

- Idso SB, Kimball BA (1992) Seasonal fine-root biomass development of sour orange trees grown in atmospheres of ambient and elevated CO<sub>2</sub> concentration. *Plant, Cell and Environment*, **15**, 337–341.
- Idso SB, Kimball BA, Mauney JR (1988) Effects of atmospheric CO<sub>2</sub> enrichment on root: shoot ratios of carrot, radish, cotton and soybean. *Agriculture Ecosystems and Environment*, **21**, 293–299.
- IPCC (1995) *Technical Summary. Inter-Governmental Panel on Climate Change*. WMO Geneva, Switzerland, 44pp.
- Jongen M, Jones MB, Hebeisen T, Blum H, Hendrey GR (1995) The effects of elevated CO<sub>2</sub> concentration on the root growth of *Trifolium repens* and *Lolium perenne* grown in a FACE system. *Global Change Biology*, **1**, 361–371.
- Kemp PR, Waldecker DG, Owensby CE, Reynolds JF, Virginia RA (1994) Effects of elevated CO<sub>2</sub> and nitrogen fertilisation pretreatment on decomposition on tallgrass prairie leaf litter. *Plant and Soil*, **165**, 115–128.
- Kimball BA, Pinter PJ Jr, Hunsaker DJ, Wall GW, Garcia RL, LaMorte RL, Wechsung G, Wechsung F, Kartschall Th (1995) Productivity and water use of wheat under free-air CO<sub>2</sub> enrichment. *Global Change Biology*, **1**, 429–442.
- Kirschbaum MUF (1995) The temperature dependence of soil organic matter decomposition and the effect of global warming on soil organic C storage. *Soil Biology Biochemistry*, **27**, 753–760.
- Lal R, Kimble J, Follett RF (1998) Pedospheric processes and the carbon cycle. In: *Soil Processes and the Carbon Cycle* (eds Lal R, Kimble J, Follett RF, Stewart BA), pp. 1–9. CRC Press, Boca Raton, FL.
- Lambers H (1993) Rising CO<sub>2</sub>, secondary plant metabolism, plant–herbivore interactions and litter decomposition. *Vegetatio*, **104/105**, 263–271.
- Leavitt SW, Paul EA, Galadima A, Nakayama FS, Danzer SR, Johnson H, Kimball BA, Tinker PB, Gregory PJ, Ingram JS, Canadell J (1996) Carbon isotopes and carbon turnover in cotton and wheat FACE experiments. *Plant and Soil*, **187**, 147–155.
- Lewin KE, Hendrey GR, Nagy J, LaMorte RL (1994) Design and application of a free-air carbon dioxide enrichment facility. *Agricultural Forest Meteorology*, **70**, 15–29.
- McMichael BL, Quisenberry JE (1993) The impact of the soil environment on the growth of root systems. *Environmental and Experimental Botany*, **33**, 53–61.
- Meyer WS, Tan CS, Barrs HD, Smith RCG (1990) Root growth and water uptake by wheat during drying of undisturbed and repacked soil in drainage lysimeters. *Australian Journal of Agricultural Research*, **41**, 253–265.
- Nagy J, Lewin KE, Hendrey GR, Hassinger E, LaMorte RL (1994) FACE facility CO<sub>2</sub> concentration control and CO<sub>2</sub> use in 1990 and 1991. *Agricultural Forest Meteorology*, **70**, 31–48.
- Norby RJ (1994) Issues and perspectives for investigating root responses to elevated atmospheric carbon dioxide. *Plant and Soil*, **165**, 9–20.
- Owensby CE, Ham JM, Knapp AK, Bremer D, Auen LM (1997) Water vapour fluxes and their impact under elevated CO<sub>2</sub> in a C4-tallgrass prairie. *Global Change Biology*, **3**, 189–195.
- Pinter PJ Jr, Kimball BA, Garcia RL, Wall GW, Hunsaker DJ, LaMorte RL (1996) Free-air CO<sub>2</sub> enrichment: Response of cotton and wheat crops. In: *Carbon Dioxide and Terrestrial Ecosystems* (eds Koch GW, Mooney HA), pp. 215–248. Academic Press, San Diego, CA.
- Prior SA, Rogers HH, Runion GB, Mauney JR (1994a) Effects of free-air CO<sub>2</sub> enrichment on cotton root growth. *Agricultural and Forest Meteorology*, **70**, 69–86.
- Prior SA, Rogers HH, Runion GB, Kimball BA (1994b) Free-Air CO<sub>2</sub> Enrichment in Cotton: Root morphological characteristics. *Journal of Environmental Quality*, **24**, 678–683.
- Prior SA, Rogers HH, Runion GB, Hendrey GR (1994c) Free-air CO<sub>2</sub> enrichment of cotton: vertical and lateral root distribution patterns. *Plant and Soil*, **165**, 33–44.
- Prior SA, Runion GB, Mitchell RJ, Rogers HH, Amthor JS (1997) Effects of atmospheric CO<sub>2</sub> on longleaf pine: Productivity and allocation as influenced by nitrogen and water. *Tree Physiology*, **17**, 397–405.
- Rogers HH, Peterson CM, McGrimmon JN, Cure JD (1992) Response of plant roots to elevated atmospheric carbon dioxide. *Plant, Cell and Environment*, **15**, 749–752.
- Rogers HH, Prior SA, O'Neill E (1993) Cotton root and rhizosphere response to free-air CO<sub>2</sub> enrichment. In: *Free-Air CO<sub>2</sub> Enrichment for Plant Research in the Field* (ed. Hendrey GR), pp. 251–263. CRC Press, Boca Raton, FL.
- Rogers HH, Runion GB, Krupa SV (1994) Plant responses to atmospheric CO<sub>2</sub> enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution*, **83**, 155–189.
- Schimel DS (1995) Terrestrial ecosystems and the carbon cycle. *Global Change Biology*, **1**, 77–91.
- Schlesinger WH (1990) Evidence from chronosequence studies for a low carbon-storage potential of soils. *Nature*, **348**, 232–234.
- Statistical Analysis Systems Institute Inc. (1985) *SAS User's Guide: Statistics*. SAS Institute Inc. Cary, NC.
- Stone LR, Teare ID, Nickell CD, Mayaki WC (1976) Soybean root development and soil water depletion. *Agronomy Journal*, **68**, 677–680.
- Thomas RB, Strain BR (1991) Root Restriction as a Factor in Photosynthetic Acclimation of Cotton Seedlings Grown in Elevated Carbon Dioxide. *Plant Physiology*, **96**, 627–634.
- Trans PP, Fung IY, Takahashi T (1990) Observational constraints on the global atmospheric CO<sub>2</sub> budget. *Science*, **247**, 1431–1438.
- van Vuuren MMI, Robinson D, Fitter AH, Chaselow SD (1997) Effects of elevated CO<sub>2</sub> and soil water availability on root biomass, root length, and N,P and K uptake by wheat. *New Phytologist*, **135**, 455–465.
- Wall GW, Kimball BA (1993) Biological database derived from free-air carbon dioxide enrichment experiments. (Design and Execution of Experiments on CO<sub>2</sub> Enrichment, Commission of the European Community, Brussels.) *Ecosystems Research Report*, **6**, 329–351.
- Wall GW, Wechsung F, Wechsung G, Kimball BA, Kartschall Th, Pinter PJ Jr, LaMorte RL (1996) Effects of Free-Air CO<sub>2</sub> Enriched (FACE) and two soil moisture regimes on vertical and horizontal distribution of root length density, surface area, and density of spring wheat. In *Annual Research Report U S Water Conservation Laboratory*, **1996**, 56–60.

- Wechsung G, Wechsung F, Wall GW, Adamsen FJ, Kimball BA, Garcia RL, Pinter PJ Jr, Kartschall Th (1995) Biomass and Growth rate of a Spring Wheat Root System grown in free-air CO<sub>2</sub> Enrichment (FACE) and ample soil moisture. *Journal of Biogeography*, **22**, 623–634.
- Wong SC, Osmond CB (1991) Elevated atmospheric partial pressure of carbon dioxide and plant growth. III. Interactions between *Triticum aestivum* C<sub>3</sub> and *Echinochloa frumentacea* C<sub>4</sub> during growth in mixed culture under different carbon dioxide nitrogen nutrition and irradiance treatments with emphasis on below-ground responses estimated using the delta carbon-13 value of root biomass. *Australian Journal of Plant Physiology*, **18**, 137–152.
- Wood CW, Torbert HA, Rogers HH, Runion GB, Prior SA (1994) Free-Air CO<sub>2</sub> Enrichment Effects on Soil Carbon and Nitrogen. *Agricultural and Forest Meteorology*, **70**, 103–116.
- Zak DR, Pregitzer KS, Curtis PS, Terri JA, Fogel R, Randlett DL (1993) Elevated atmospheric carbon dioxide and feedback between carbon and nitrogen cycles. *Plant and Soil*, **151**, 105–117.